ELSEVIER

Journal of Chromatography A, 680 (1994) 675–684

JOURNAL OF CHROMATOGRAPHY A

Determination of anions with capillary electrophoresis and indirect ultraviolet detection

M.M. Rhemrev-Boom

CASE Lab, Van Schaikweg 94, 7811 KL Emmen, Netherlands

Abstract

A method is validated for the determination of anions with capillary electrophoresis (CE) in combination with indirect UV detection. The method described here is used for the analysis of eight of the most common anions (fluoride, chloride, bromide, sulphate, nitrate, nitrite, thiosulphate and phosphate). Next, the method is compared with a another buffer system for the determination of anions with CE and indirect UV detection. Typical limits of detection are obtained between 1 and 3 mg/l for the above-mentioned compounds. The repeatability and reproducibility of the system differs per compound and is, with the exception of fluoride and phosphate, between 4 and 6% and 5–10%, respectively. Linearity was observed between 1 and 10 mg/l. The method is applied for the determination of anions in drinking water, serum and urine.

1. Introduction

Today, the determination of ionic species is generally carried out by ion chromatography (IC), which uses the electrochemical properties of the analytes, such as ionic interactions and conductivity. The ability to separate and detect several widely different ionic species simultaneously is an unique characteristic of IC. However, there are important limitations to IC, including the lack of sufficient selectivity, low separations efficiency and relatively large elution volumes.

Capillary electrophoresis (CE) is an efficient analytical separation technique for analysis of minute amounts of sample, and has several advantages, including improved resolution and fast separations.

Long before the development of modern CE techniques, Mikkers et al. [1] described the separation of anions by electrophoresis in small tubes. Since then, several groups of investigators described the use of CE for the analysis of anions [2–14]. Some used conductivity for the detection of the anions [5,6]; others developed indirect laser-induced fluorescence detection systems [3]. Indirect UV detection has been used successfully for the determination of anions in different matrices [8,10,12–14]. In this case separation is obtained with an electrolyte containing an UV absorbing anion plus an electroosmotic flow modifier. The displacement of the chromophore by the analyte ions permit indirect photometric detection. By adding a so-called electroosmotic modifier (a cationic surfactant) the direction of the electroosmotic flow can be reversed, forcing the anions to migrate in the same direction as the electroosmotic flow from the injection end toward the detection end. Secondly, by selecting an electrolyte anion with a mobility that is closely matched to the ions to be determined, separations could be achieved rapid-

0021-9673/94/$07.00
SSDI 0021-9673(94)00676-8

ly within several minutes. Based on this principle, Fluka Chemie AG composed an electrolyte, in which pyromellitic acid was chosen as chromophore and hexamethonium hydroxide as electroosmotic modifier. As already shown by Dionex [15] it is possible to separate the most common inorganic anions with this buffer solution.

This article describes the validation of the determination of eight common anions (fluoride, chloride, bromide, sulphate, nitrite, nitrate, thiosulphate and phosphate) with the buffer system delivered by Fluka Chemie and the Model PRINCE CE apparatus. Next, the method is compared with a home-made buffer solution, containing chromate as chromophore and tetradecyltrimethylammonium bromide (TTAB) as electroosmotic flow modifier, and a (similar) commercial buffer solution (Dionex).

2. Experimental

2.1. Instrumentation

A PRINCE CE instrument (Lauer Labs, Emmen, Netherlands) in combination with a Model 759A absorbance detector (Separations, Hendrik Ido Ambacht, Netherlands) was used. Data were collected with the Gyncosoft chromatography data system (Separations). The column used was a polyimide-coated, 60 cm × 50 μm I.D. fused-silica capillary (Thermo Separation Products). The injection mode was hydrodynamic. Operating conditions are provided in the captions to the figures.

2.2. Reagents

All solutions were prepared using demineralised water (Water Treatment Rossmark-Van Wijk and Boerma, Almelo, Netherlands). Sodium chromate and TTAB were obtained from Fluka (Buchs, Switzerland). Boric acid (p.a.) was obtained from Janssen (Beerse, Belgium). Anion standards used were prepared from analytical sodium salts purchased from Brunschwig Chemie (Amsterdam, Netherlands).

2.3. Electrolyte solutions

The electrolyte solution obtained from Fluka and Dionex (Breda, Netherlands) basically consists of 2.25 m*M* pyromellitic acid, 6.5 m*M* sodium hydroxide, 0.75 m*M* hexamethonium hydroxide and 1.6 m*M* triethanolamine, pH 7.7 ± 0.2 (information provided by Fluka and Dionex, respectively).

The home-made electrolyte solution was made by bringing the pH of a 5 m*M* boric acid solution to pH 8.0 with 0.2 *M* sodium hydroxide. To this solution 5 ml of a 0.1 *M* sodium chromate solution and 0.5 ml of a 0.1 *M* TTAB solution was added. The pH of the electrolyte buffer was checked prior to use, and, if necessary, brought to pH 8.

3. Results and discussion

3.1. CE configuration

Normally, sample introduction is carried out at the anodic and detection at the cathodic electrode. The electroosmotic flow (EOF), a bulk fluid flow which appears at a pH > 2, is toward the cathode and is dependent on the charge on the capillary wall. By introducing cationic surfactants into the buffer, the direction of the EOF is reversed. For this purpose, injection is thereby carried out at the cathodic electrode and detection at the anodic electrode. Due to the fact that inorganic anions have a large charge-to-size ratio, these species migrate toward the anode preceding the EOF. This finally results in a very efficient and short analysis.

With respect to the injection technique in CE, in this case two modes can be chosen: hydrodynamic and electrokinetic injection. The first method is used exclusively in this work. By using a pressure differential, based on Boyle's law, a representative sample of a homogeneous mixture is introduced into a capillary [16].

Analysis is carried out as described above. Detection occurs by the process of indirect photometry at 250 nm (for the Fluka and Dionex

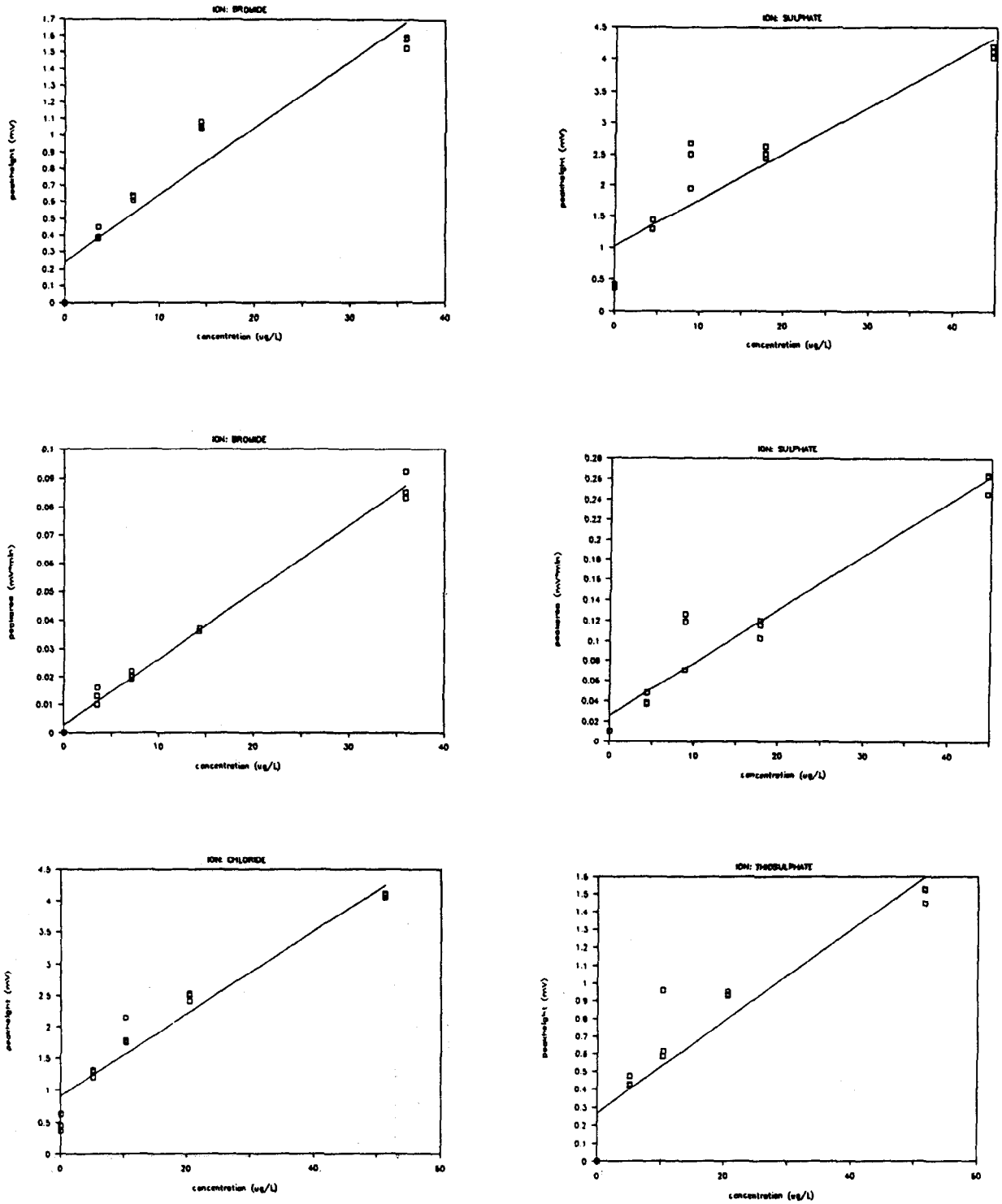

Fig. 1. *(Continued on p. 678)*

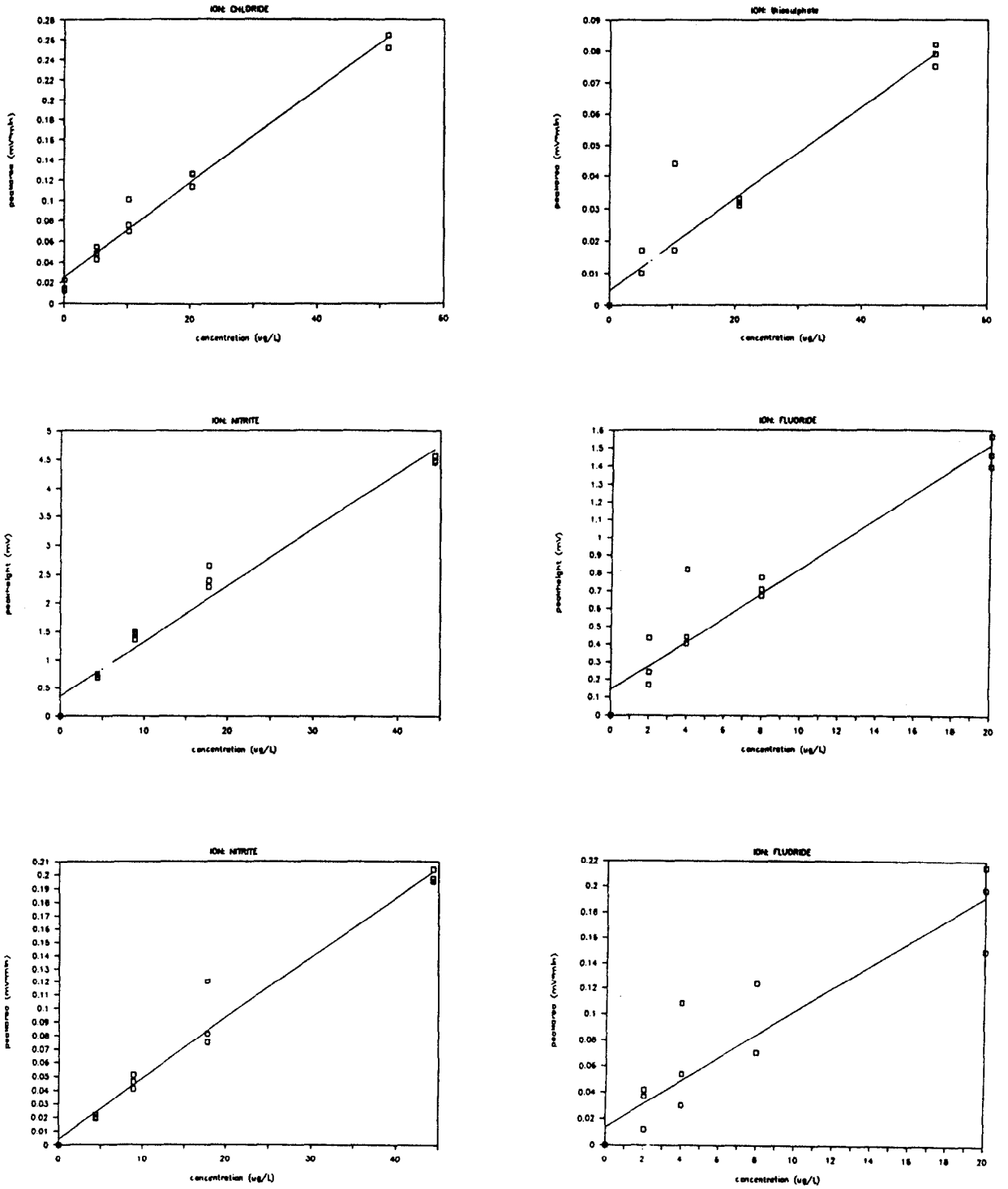
ION: CHLORIDE
peakheight (mV)
concentration (ug/L)
ION: thiosulphate
peakheight (mV)
concentration (ug/L)
ION: NITRITE
peakheight (mV)
concentration (ug/L)
ION: FLUORIDE
peakheight (mV)
concentration (ug/L)
ION: NITRITE
peakheight (mV)
concentration (ug/L)
ION: FLUORIDE
peakheight (mV)
concentration (ug/L)

Fig. 1.

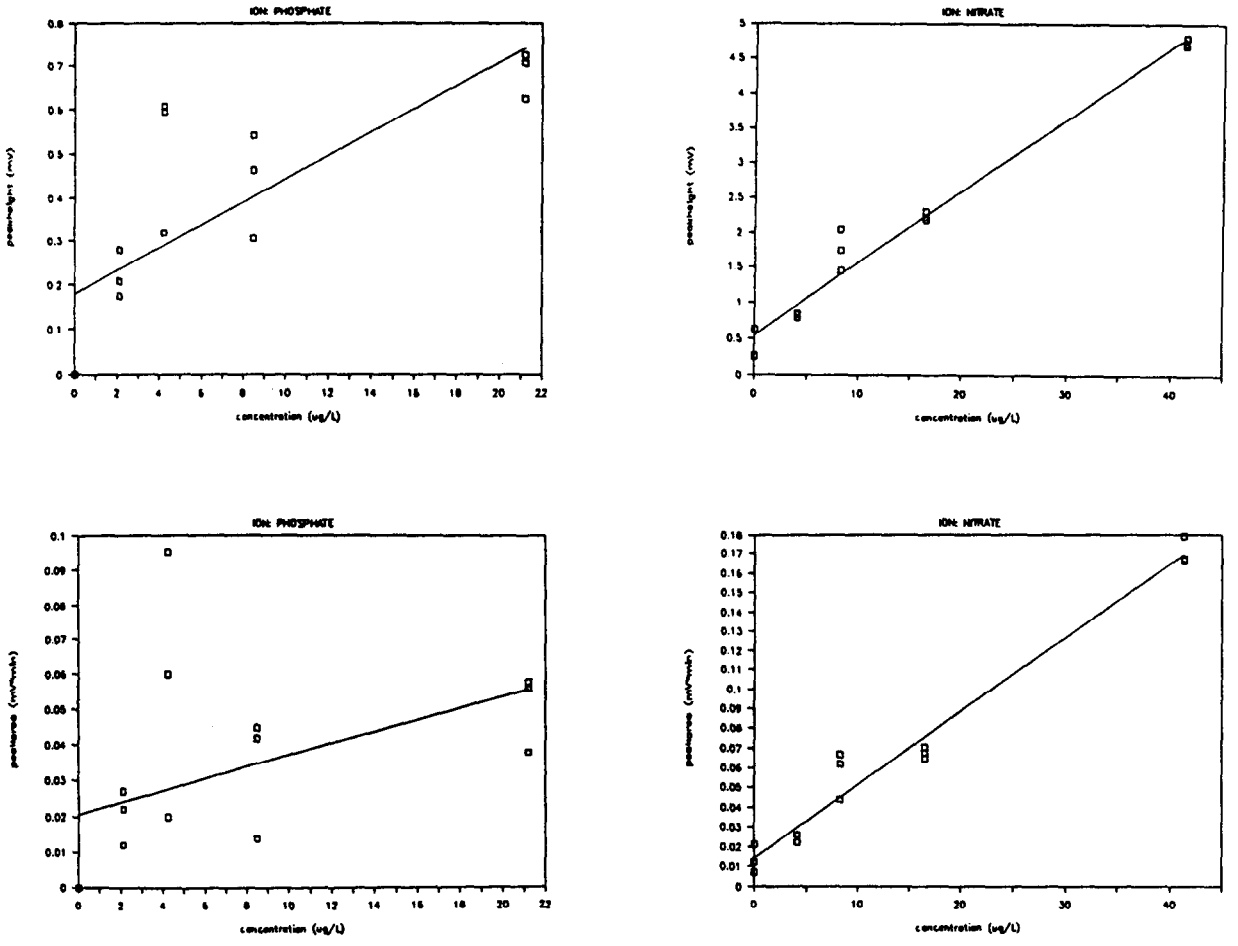

Fig. 1. Calibration plots of eight anions (peak heights or peak areas versus the concentration per anion mg/l).

buffer solutions) and at 272 nm (for the home-made buffer solution).

3.2. Validation of the Fluka buffer

Prior to analysis, the capillary was washed with a 2 *M* hydrochloric acid solution followed by a 0.2 *M* sodium hydroxide solution. When applying the buffers from Fluka or Dionex, the capillary was equilibrated during half an hour at 30 kV. For the home-made buffer solution, a longer equilibration time was required (approximately one day).

For the validation of the Fluka buffer, a stock solution was prepared in water containing a mixture of inorganic ions (thiosulphate, bromide, chloride, sulphate, nitrite, nitrate, fluoride and phosphate) with a concentration of approximately 200–500 mg/l per compound. Dilutions were made in water from the stock solution, and every standard plus a blank (demineralised water) was injected at least three times. Based on the measured peak heights (mV) or peak areas (mV·min), calibration plots were calculated with linear regression analysis. The results are presented in Fig. 1. For most of the components good linearity can be observed at concentrations between 1 and 10 mg/l. At higher concentrations a significant deviation from a calculated first order calibration plot can clearly be seen and a second order correction should be

Table 1
LOD[a], the repeatability[b] and reproducibility[b] of the migration time, peak height and peak area for eight anions determined with capillary electrophoresis and indirect UV-detection using the Fluka buffer (data are based on a 50 mbar hydrodynamic injection during 6 s)

Compound	LOD (mg/l)	Repeatability (%R.S.D.), $n = 9$			Reproducibility (%R.S.D.), $n = 12$		
		M.T.	P.H.	P.A.	M.T.	P.H.	P.A.
$S_2O_3^{2-}$	2	0.09	6	12	0.6	6	30
Br^-	3	0	6	15	0.5	9	23
Cl^-	1	0.1	5	11	0.7	5	12
SO_4^{2-}	1	0.1	4	11	0.7	6	15
NO_2^-	3	0.08	4	10	0.7	6	11
NO_3^-	2	0.07	4	7	0.6	7	10
F^-	2	0.2	29	67	0.4	48	53
HPO_4^{2-}	1	2	31	30	1	32	56

[a] LOD is defined as three times the noise and is expressed as mg/l anion. The noise being 0.2 mV (rise time detector 1 s).
[b] Demonstrated are the relative standard deviations in the migration time (M.T.), peak height (P.H.) and peak area (P.A.).

applied. Due to large deviations (see Table 1) in the analysis of fluoride and phosphate, bad correlations are observed with respect to regression analysis.

In order to determine the repeatability, a standard mixture of the above mentioned components with a concentration of approximately 5 mg/l per compound was injected nine times. The relative standard deviations (R.S.D.s) in the peak heights, peak areas and migration times of the anions were determined. The reproducibility, expressed as the R.S.D. of the migration times, peak heights and peak areas, was determined by analyzing the same standard on different days. Next, limits of detection (LODs), defined as three times the noise, were determined for each component. The results for the repeatability, reproducibility and LOD for the Fluka buffer are given in Table 1.

For comparison, the repeatability and reproducibility of the migration times, peak heights and the LODs of the above-mentioned anions using the home-made electrolyte solution was determined in the same manner. The results for this electrolyte solution are given in Table 2. Typical electropherograms for both systems are shown in Figs. 2 and 3, respectively.

As can be seen from Table 1 the migration times for the eight anions when using the Fluka electrolyte buffer seem to be sufficiently repeatable and reproducible. With respect to the home-made buffer, the repeatability as well as the reproducibility of the migration times was found to be significantly worse compared to those when using the Fluka buffer. With the exception of fluoride and phosphate, the repeatability and reproducibility of the measured peak heights are well in line with conventional (ion) chromatographic techniques. However, with respect to the measured peak areas large deviations were observed (see Table 1). This can be explained by the fact that the integration program used showed difficulties in drawing accurate baselines for negative peaks and had to be done by hand. Therefore, comparison with the home-made buffer is done by measuring and comparing the peak heights only. With respect to the peak heights comparable results were obtained, with the exception of those for fluoride and phosphate.

As demonstrated in Fig. 2, the peaks for fluoride and phosphate, which can be defined as relatively slow migrating components, are not symmetric and relatively broad compared to the other components. In contrary, better peak-

Table 2
LOD[a], the repeatability[b] and reproducibility[b] of the migration time and peak height for eight anions determined with capillary electrophoresis and indirect UV-detection using the home-made buffer (data are based on a 50 mbar hydrodynamic injection during 6 s)

Compound	LOD (mg/l)	Repeatability (%R.S.D.), $n = 9$		Reproducibility (%R.S.D.), $n = 12$	
		M.T.	P.H.	M.T.	P.H.
$S_2O_3^{2-}$	0.6	0.8	4	2	8
Br^-	0.6	0.8	7	2	14
Cl^-	0.1	0.8	3	2	4
SO_4^{2-}	0.2	1	3	2	6
NO_2^-	0.6	1	3	2	6
NO_3^-	0.4	0.8	6	3	7
F^-	0.8	0.9	4	2	18
HPO_4^{2-}	2	0.7	7	3	29

[a] LOD is defined as three times the noise and is expressed as mg/l anion. The noise being 0.005 mV (rise time detector 1 s).
[b] Demonstrated are the relative standard deviations in the migration time (M.T.) and peak height (P.H.).

shapes were performed using the home-made buffer (see Fig. 3). This probably explaines the fact that for fluoride and phosphate better results were obtained when using the home-made buffer. For the fast migrating components, however, the Fluka buffer seems to be more suitable. During the primary experiments with the Fluka buffer, it was found that separation between these species remained even at high injection volumes (100 nl) and/or if one of these components was added in excess (which is frequently the case with samples). This adverse phenomena was noticed by us when using the home-made buffer.

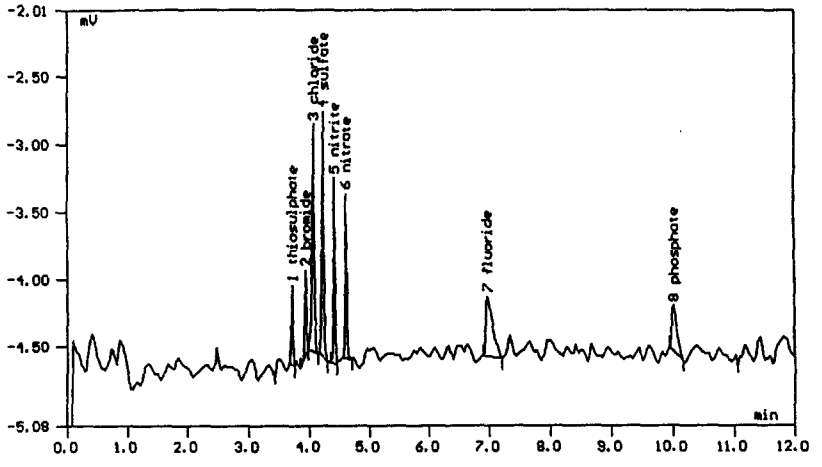

Fig. 2. Electropherogram of standard anions. Capillary 60 cm × 50 μm I.D. fused-silica. Electrolyte: Trace anion buffer (Fluka). Injection: hydrodynamic, 50 mbar, 6 s, Potential: 20 kV. Detection: indirect 250 nm. Solutes: 1 = $S_2O_3^{2-}$ (5 mg/l); 2 = Br^- (5 mg/l); 3 = Cl^- (6 mg/l); 4 = SO_4^{2-} (7 mg/l); 5 = NO_2^- (6 mg/l); 6 = NO_3^- (6 mg/l); 7 = F^- (2 mg/l); and 8 = HPO_4^{2-} (1 mg/l).

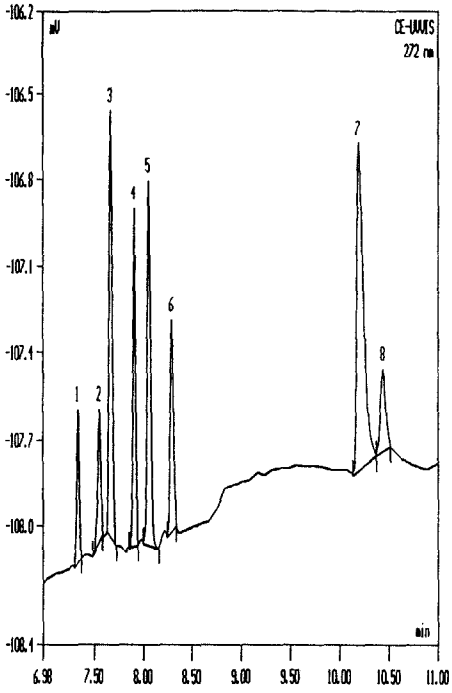

Fig. 3. Electropherogram of standard anions. Capillary 73 cm × 75 μm I.D. fused-silica. Electrolyte: 5 m*M* sodium chromate, 0.5 m*M* TTAB, 5 m*M* boric acid, pH 8.0 Injection: hydrodynamic, 20 mbar, 6 s, Potential: 20 kV. Detection: indirect 272 nm. Solutes: 1 = $S_2O_3^{2-}$ (4 mg/l); 2 = Br^- (6 mg/l); 3 = Cl^- (8 mg/l); 4 = SO_4^{2-} (6 mg/l); 5 = NO_2^- (7 mg/l); 6 = NO_3^- (7 mg/l); 7 = F^- (5 mg/l); and 8 = HPO_4^{2-} (2 mg/l).

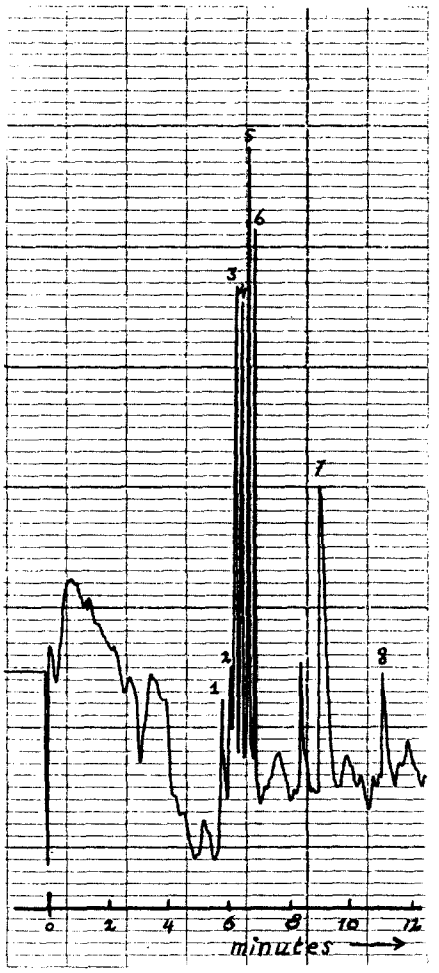

Fig. 4. Electropherogram of standard anions. Capillary 73 cm × 75 μm I.D. fused-silica. Electrolyte: Ionphor Anion PMA (Dionex) Injection: hydrodynamic, 10 mbar, 6 s, Potential: 30 kV. Detection: indirect 250 nm. Solutes: 1 = $S_2O_3^{2-}$ (11 mg/l) 2 = Br^- (12 mg/l); 3 = Cl^- (21 mg/l); 4 = SO_4^{2-} (15 mg/l); 5 = NO_2^- (36 mg/l); 6 = NO_3^- (18 mg/l); 7 = F^- (13 mg/l); and 8 = HPO_4^{2-} (5 mg/l).

As can be seen in Fig. 2, the noise in the baseline when using the Fluka buffer is relatively high (0.2 mV compared to 0.005 mV for the home-made buffer). This explains the high LOD determined for the eight anions compared to those of the home-made buffer. Comparable electropherograms (see Fig. 4) were obtained when using the Dionex buffer; in this case a baseline with a relatively high noise level was also noticed. Filtering the buffer with a 0.45-μm filter did not show any positive effect. Without further experiments on this subject, no explanation can be given for this phenomena.

Next, the Fluka buffer was applied for the determination of anions in three different matrices: tap water, urine and serum. With respect to tap water, direct injection was possible. The urine sample was diluted 50 times prior to analysis. Due to the fact that proteins, present in serum, tend to adsorb to the capillary wall and thereby influencing the migration times, they were removed prior to analysis. This was done by means of spinning 0.5 μl sample in a Microcon-3 concentrator (Grace, Amicon Division, MW cutoff 3000) at 10 000 *g* for 20 min. The filtrate obtained was diluted 25 times and

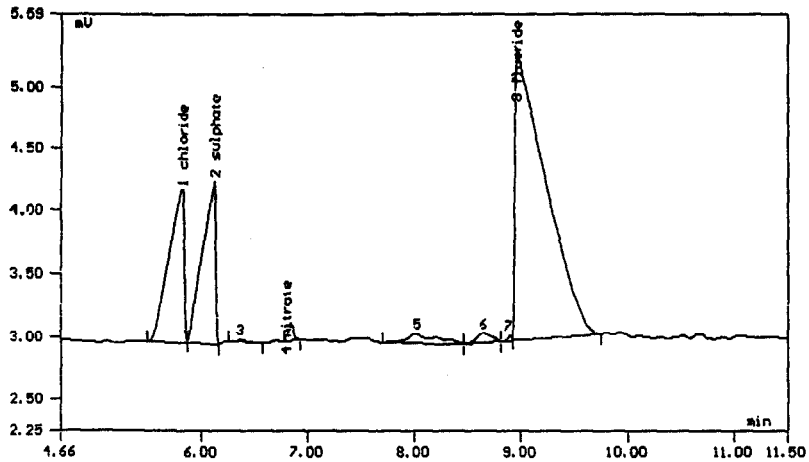

Fig. 5. Electropherogram of tap water. Capillary 60 cm × 50 μm I.D. fused-silica. Electrolyte: Trace anion buffer (Fluka). Injection: hydrodynamic, 50 mbar, 6 s, Potential: 20 kV. Detection: indirect 250 nm. Solutes: 1 = chloride (38 mg/l); 2 = sulphate (52 mg/l); 4 = nitrate (4 mg/l); 8 = fluoride (44 mg/l).

was ready for use. Electropherograms of tap water, urine and serum are demonstrated in Figs. 5, 6 and 7, respectively.

For human urine and serum, reference values were given by clinical chemists for chloride of 110–250 mmol and 101–111 mmol, respectively. For urine and serum, we analyzed a concentration of 184 m*M* and 130 m*M*. Based on these limited results, this method apparently shows possible potential for the application of CE in clinical laboratories. However, a more severe study on this subject has to be performed before introducing the technique in this area.

4. Conclusions

In this work the electrolyte solution from Fluka Chemie AG is validated and compared with other available electrolyte solutions for the

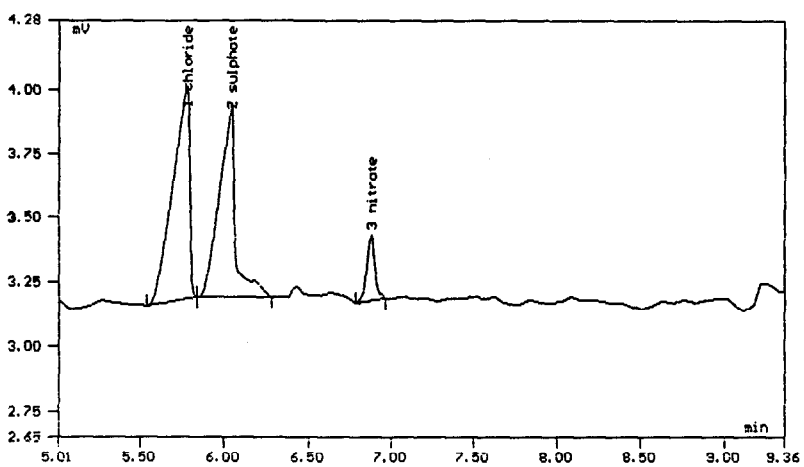

Fig. 6. Electropherogram of diluted urine. Capillary 60 cm × 50 μm I.D. fused-silica. Electrolyte: Trace anion buffer (Fluka). Injection: hydrodynamic, 10 mbar, 6 s, Potential: 20 kV. Detection: indirect 250 nm. Solutes: 1 = chloride (130 mg/l); 2 = sulphate (100 mg/l); 3 = nitrate (31 mg/l).

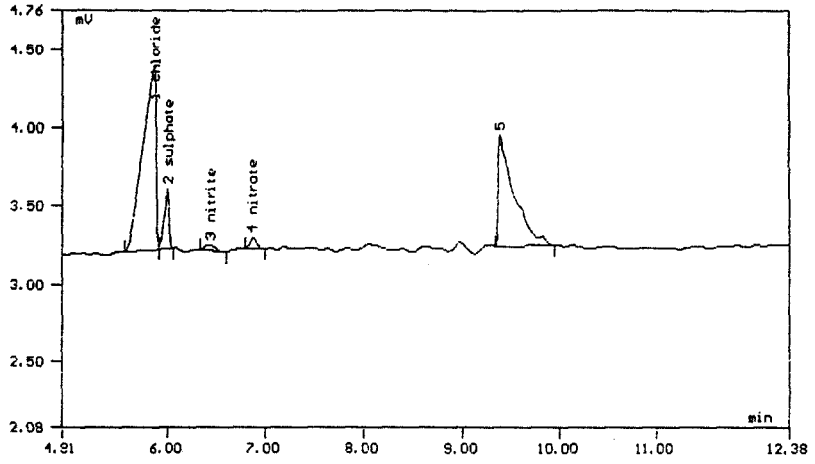

Fig. 7. Electropherogram of diluted serum. Capillary 60 cm × 50 μm I.D. fused-silica. Electrolyte: Trace anion buffer (Fluka). Injection: hydrodynamic, 10 mbar, 6 s, Potential: 20 kV. Detection: indirect 250 nm. Solutes: 1 = chloride (180 mg/l); 2 = sulphate (52 mg/l); 4 = nitrate (9 mg/l); 5 = not identified.

determination of inorganic anions with the Model PRINCE (Lauer Labs) CE instrument in combination with indirect UV detection. The system is suitable for the determination of fast migrating anions, such as thiosulphate, bromide, chloride, sulphate, nitrite and nitrate.

Although the method seems to be sufficient repeatable and reproducible for the determination of these inorganic anions, the method lacks in linearity. With regard to the repeatability and reproducibility, comparable results were obtained with another, home-made, electrolyte solution.

To increase the potential of the method, better LODs should be obtained. In order to decrease the noise in the baseline, more work is in progress with respect to the composition of the electrolyte buffer.

References

[1] F. Mikkers, F. Everaerts and T. Verheggen, *J. Chromatogr.*, 169 (1979) 11.
[2] W.G. Kuhr and E.S. Yeung, *Anal. Chem.*, 60 (1988) 2642.
[3] L. Gross and E.S. Yeung, *J. Chromatogr.*, 480 (1989) 169.
[4] Th.P.E.M. Verheggen, A.C. Schoots and F.M. Everaerts, *J. Chromatogr.*, 503 (1990) 245.
[5] F. Foret, S. Fanali, L. Ossicini and P. Bocek, *J. Chromatogr.*, 470 (1989) 299.
[6] J. Beckers, T. Verheggen and F. Everaerts, *J. Chromatogr.*, 452 (1988) 591.
[7] S. Terabe and T. Isemura, *Anal. Chem.*, 62 (1990) 650.
[8] T. Romano, P. Jandik, W.R. Jones and P.E. Jackson, *J. Chromatogr.*, 546 (1991) 411.
[9] J. Sudor, J. Pospichal, M. Deml and P. Bocek, *J. Chromatogr.*, 545 (1991) 331.
[10] W.R. Jones and P. Jandik, *J. Chromatogr.*, 546 (1991) 445.
[11] X. Huang, J.A. Luckey, M.J. Gordon and R.N. Zare, *Anal. Chem.*, 61 (1989) 766.
[12] P. Jandik, W.R. Jones, A. Weston and P.R. Brown, *LC·GC*, 9 (1991) 634.
[13] B.J. Wildman, P.E. Jackson, W.R. Jones and P.G. Alden, *J. Chromatogr.*, 546 (1991) 459.
[14] W.R. Jones and P. Jandik, *J. Chromatogr.*, 608 (1992) 385.
[15] *Application Note 68*, 1992, Dionex B.V., Breda, Netherlands.
[16] H.H. Lauer, J.B. Ooms, B.H. Nieland, P. Waardenburg, presented at the *International Symposium on High Performance Capillary Electrophoresis, Amsterdam, 9–13 February, 1992*, T42.